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Full Length Research Paper

Compositions and comparisons of antimicrobial potencies of some essential oils and antibiotics against selected bacteria

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The antimicrobial activities of 10 essential oils extracted from various plant species were investigated and compared with the activities of 10 commercial antibiotics against 10 strains of bacteria using agar diffusion method. Although, all the essential oils were active at concentration ranging from 0.5 to 1.5 mg/ml, their activities were more lower than the commercial antibiotics. However, being natural products the oils have been reported to be much safer than the antibiotics. Another advantage of the essential oils used in this study was their broad spectrum activities against gram positive and gram negative bacteria. The oils were analyzed by GC and GC-MS techniques in order to determine their active compounds.

Key words: Essential oils, antibiotics, GC-MS, antimicrobial activities.

INTRODUCTION

The widespread and injudicious use of antibiotics has resulted in the emergence of antibiotic-resistant pathogens. Consequently, it has posed a serious threat to global public health (WHO, 2002). It is generally accepted that unnecessary use of antibiotics is a major promoter of the emergence and transmission of drug-resistant bacterial strains. The resistance which certain microorganisms have developed against antibiotics has prompted investigation of antimicrobial activities and different application of plant essential oils against a wide range of Gram positive and Gram negative bacteria including antibiotic resistant species. Many plants and their essential oils have also been reported to have potential application in medical procedures as well as in the pharmaceutical, cosmetic and food industries (Jones, 1996). Thus, plant essential oils constitute a possible natural alternative to chemical-based bactericides which might affect food safety and preservation (Lanciotti et al., 2003).

The antimicrobial activity of essential oils is assigned to the presence of a number of small terpenoids and phenolic compounds (thymol, carvacrol, eugenol, α-terpineol, γ-terpinene), which in pure form demonstrate high antibacterial activity (Brannan, 1996). There is evidence that essential oils are more strongly antimicrobial than is accounted for by the additive effect of their major antimicrobial components, thus suggesting a significant role for minor components (Lattaoui and Tantaoui-Elaraki, 1994). Essential oils and their components in this study are observed to be active against bacteria, including gram negative and gram positive bacteria which is in contrast to the report of Zaika (1988) that gram positive bacteria were more resistant to essential oils than gram-negative bacteria. Also in contrast to the hypothesis proposed by Deans and Ritchie (1987) that the gram negative bacteria were more resistant than gram positive due to the presence of lipopolysaccharides in their outer membrane.

The objective of the present study was to investigate antimicrobial activity of plant essential oils and convectional antibiotics against a diverse range of both gram positive and gram negative bacteria, thus genera- ting quantitative antimicrobial data and to generate data for oils for which little data exist.

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MATERIALS AND METHODS

Bacteria strains used in this study

The following gram positive bacteria: *Bacillus cereus* (ATCC 10876), *Bacillus subtilis* (ATCC 6051), *Listeria monocytogenes* (ATCC 12022), *Streptococcus pyogenes* (ATCC 19615); and gram negative bacteria: *Escherichia coli* (ATCC 87536), *Klebsiella pneumoniae* (ATCC 13883), *Proteus vulgaris* (ATCC 43071), *Pseudomonas fluorescens* (ATCC 13525), *Shigella flexneri* (ATCC 12022) and *Alcaligenes faecalis* (ATCC 8750) were used in this study. The organisms were sub-cultured in nutrient broth and nutrient agar for both essential oils and antibiotics susceptibility testing. They were chosen based on their pathological effects on humans and their role in the deterioration of food products. All the strains were obtained from the Institute of Louis Pasteur, Paris (France).

Essential oils

Four plant species; *Pteronia incana, Artemisia afra, Lavandula officinalis* and *Rosemary officinalis* were collected from the Eastern Cape Province, South Africa. The essential oils were extracted by hyrodistillation in the department of Biochemistry and Microbiology, University of Fort Hare. Six commercial essential oils includes *Tagetes minuta, Citrus limon, Citrus sinensis, Eriocephalatus punchulatus, Eucalyptus cinerea* and *Ocimum basil* used in this study were purchased from Lung Kai Fook Medical and Co. Ltd (Hong Kong).

Antibiotics

The antimicrobial activity of 10 commercial antibiotics was carried out using selected bacteria strains. The antibiotics used were chloramphenicol, penicillin G, tetracycline, streptozotocin, rifampicin, norfloxacin, actinomycin D, chromomycin A_3, arugomycin and netropsin all purchased from Sigma-Aldric (USA).

Culture Media and growth conditions

The bacterial stock cultures were maintained on nutrient agar (Saarchem, Gauteng, SA) plates. A loopful of bacterial cells from the nutrient agar plates was inoculated into 100 ml nutrient broth (Difco, California, USA) in 250 ml side arm Erlenmeyer flask and incubated at 37°C for 16 h with vigorous shaking (orbital incubator, S150, UK). After incubation, the culture was diluted with fresh media to give an $O.D_{600nm}$ of 0.1. One hundred microliters of the culture cells was added onto the plate and spread into a bacterial lawn using a sterile glass spreader.

Preparation of working solution of essential oils and antibiotics

Essential oils (neat) were diluted with Tween- 80 (used as control) to give a working concentration ranging between 0.01- 2 (mg/ml). This range was used for the determination of Minimum Inhibitory Concentration (MIC). The desired concentration of chloramphenicol and rifampicin was prepared in methanol (100 % w/v), tetracycline was dissolved in ethanol (95% w/v); penicillin, amoxicillin, ampicilin, netropsin and actinomycin D were dissolved in sterile distilled water to obtain various concentrations for MIC determination. Aliquots of stock solutions (5 ml) were dispensed into microfuge tubes and frozen at 0°C. The stock solutions were prepared using the formula (1000/P) × V × C = W, where P stands for the potency of the antibiotic base, V = volume in ml required, C = final concentration of the antibiotics and W = weight of the antibiotic to be dissolved in volume (V) (NCCLS, 2002).

Susceptibility test

The agar well diffusion test based on the method of Deans and Ritchie (1987) was used to determine the susceptibility of bacteria. Precisely, 100 µl of standardized 18 h bacterial cultures were spread on nutrient agar plate to create a bacterial lawn. The cultures were standardized to approximately 10^5 CFU /ml using McFarland standard. The absorbance of 0.1 at 600 nm was taken for the standardization. Wells with diameter of 4 mm were punched in each nutrient agar plate and 25 µl of various concentrations of the essential oils and antibiotics were added separately to the wells under aseptic condition. The plates were left for 30 min at room temperature for the diffusion of the essential oils and antibiotics before being incubated at 37°C for 18 h. The agar well method used in this study was limited in that some substances in the agar were inadequately diffused causing an uneven zone of inhibition. The diameters of the zones of inhibition were measured after 18 h. All analyses were carried out in triplicates.

Determination of minimum inhibitory concentration of antibiotic and essential oils

MIC of antibiotics and essential oils were determined by agar diffusion method (Irobi et al., 1996) using two- fold increment (0.016 to 512 µg/ml) while essential oils were diluted with Tween-80 to give concentrations ranging from 0.01 to 2 mg/ml (w/v) on nutrient agar plates seeded with approximately (10^5 CFU/ml) of test organisms and were incubated at 37°C overnight. Fifty microlitre of standardized 18 h incubated bacterial culture was introduced into test tubes, followed by the addition of varying concentration of essential oils and antibiotics. The MIC was recorded as the lowest concentration that inhibits the growth of the bacterial strains. Tween-80 was used as the negative control in essential oils but showed minimal effect on the test bacteria.

GC- MS analysis

Analysis of the components of the essential oils was carried out using GC-MS (HP 6890) with a mass selective detector (HP5973). Identification of the components of essential oils was accomplished by comparison with the standards available in the database. The quantity of compounds was calculated by integrating the peak areas of spectrograms. A needle with the sample material (essential oils tested) was inserted directly into the inlet of a Hewlett Packard (HP 6890, USA) Gas Chromatograph. The temperature of the injection port was maintained at 220°C while the pressure at the inlet was maintained at 3.96 psi. A HP-5 MS (crosslinked 5% Phenyl Methyl Siloxane) column (30 m × 0.25 mm × 0.25 µm film thickness) was temperature- programmed from 60 to 150°C at 3°C min^{-1} after a 3 min delay. Helium was used as a carrier gas at 0.7 ml min^{-1}. Mass spectra were recorded by a 5973 series Mass Selective Detector (MSD) (Adam, 2001).

RESULTS AND DISCUSSION

The main components of the essential oils identified and their retention indices by the GC-MS analysis are given in Tables 1 and 2 while the results for the antibacterial activity tests of essential oils and antibiotics are given in Tables 3 and 4, respectively.

Chemical analysis of essential oils used in this study revealed about 12 to 23 components with significant quantitative differences in the oils composition. Most of the oils contained at least 8 different chemical compounds.

The components of the oils analysed were unique to certain essential oils such as: β-thujone was only found in *A. afra* oil; propanoic acid in *Eriocephalus punchulatus* oil; estragole in *O. basilicum* oil; P-cymene in *P. incana* oil; dihydrotagetone, δ-4-carene and tagetone were found only in *T. minuta* oil.

P. incana oil displayed the highest number of constituents with 23 detected compounds and showed the highest content of p-cymene (19.15 %), and lower content of 2(E)(pentenyl)-buten-1,4-diol. Similar observations have been made elsewhere (Mangena and Muyima, 1999) with the exception that, o-cymene, α-pinene and β-pinene components were not found in our study.

Twenty-two constituents were identified in *O. basilicum* oil with highest content of estragole (68.43%) while other studies have reported linalool and methyl chavicol as the highest major components with antimicrobial potency (Ntezurubanza et al., 1984).

E. cinerea showed 23 detected compounds. The compound 1, 8 Cineole was the most abundant constituent in the oil (77.8%) followed by α- terpineol (2.1%) and β-Eudesmol (2.05%). Although, 1, 8 cineole was the most abundant component, a major contributor for the bioactivity was assumed to be α- terpineol (Zakarya et al., 1993).

The essential oil of *L. officinalis* was found as the least complex of all the oils analysed and contained 12 detected compounds, with a high content of camphor (16.28%) followed by endoborneol (8.85%). *T. minuta* oil contained δ-4-carene (30.1%) as the dominating compound which is not found in other essential oils. Phenol and terpineol-4-ol were among its major components and have been reported to possess antibacterial activities (Cosentino et al., 1999).

Eighteen components in *A. afra* were identified with β-thujone (30.3%) constituting the highest percentage and tetrahydroionone (0.43%) constitutes the lowest composition. Camphor was also detected in this study contrary to other reports while α- thujone was missing contrary to the report by Mangena and Muyima (1999). The analysis results differ, according to literature data, as far as the major compounds are concerned.

The GC-MS analysis of *C. limon* oil revealed the presence of limonene as the dominant compound and this corroborates a previous report by Dorman and Deans (2000).

R. officinalis oil was shown to contain the highest composition of Bornyl acetate (4.85%) and Camphor (3.85%). This oil has been reported to have antibacterial activity due to the presence of camphor (Graven et al., 1992).

For the *C. sinensis* oil, a total of eighteen compounds were identified, representing about 95.2% of the total oil composition. The main constituents were found to be dominated by limonene (70.16%). Among other major components were linalool (3.31 %), α -terpineol (2.1 %) and decanal (2.63 %). The monoterpenes (α-terpineol, carveol, camphor and linalool) and sesquiterpenes (valencene, and humulene) components of this oil have high enough vapour pressure at normal atmospheric condition to allow for their significant release into the air in order to enhance their antibacterial activity (Dudareva et al., 2004).

The main components of *E. punchanlatus* were found to be dominated by propanoic acid with the highest percentage composition of (30.15%). The other major compounds were butanoic acid (7.75%), butenoic acid (6.94%), acetic acid (4.8%), acetoxylinalool (3.25%), l-nonene (2.49%) and cyclohexane (3.1%). Some other major components have not been found in our studies but have been reported elsewhere. These include chamazulene, α-pinene, 2-methylbutanol and terpinene-4-ol (Njenga et al., 2005). The overall, chemical composition indicates all essential oils used in this study have a higher diversity of chemical compounds except *L. officinalis*.

The results of the antibacterial tests indicated that the essential oils tested have a broad spectrum of inhibitory activity. *B. cereus* and *Proteus vulgaris* showed highest sensitivity to the oils of *E. punchanlatus, O. basilicum, C. limon* and *C. sinensis* (Table 3). *S. pyogenes, P. vulgaris, K. pnuemoniae* and *A. faecalis* also displayed highest sensitivity to the oil of *A. afra* and *L. officinalis*. Some of the bacteria showed a significant and weak sensitivity to the oils tested at MIC between 1.0 to 1.5 mg/ml as shown in Table 3.

P. incana oil displayed a fairly broad spectrum of antibacterial activity, particularly at high concentrations. *T. minuta, A. afra, R. officinalis, L. officinalis. E. cinerea, P. incana and T. minuta oils* displayed similar antibacterial activity *while C. sinensis, C. limon* and *E. punchanlatus* showed strong broad spectrum antibacterial activity at a low MIC as shown in Table 3. In the present study, all the essential oils tested were effective against gram positive and gram negative bacteria (0.5 to 1.0 mg/ml w/v). It is very interesting to note that the oils showed antimicrobial activity against known food pathogens such as *L. monocytogenes, B. cereus, E. coli* and *P. vulgaris* which might be the cause of food spoilage and poisoning and subsequently cause human diseases.

The results obtained might be different from previous reports due to the differences in oil composition which could probably be attributed to the different genotypes of the plant used, seasonal variation, as well as the method of extraction of the oil and/or environmental factors. The observed differences might also be due to different chemotypes and the nutritional status of the plants (Lachowicz et al., 1996). Some oils with the same common name may also be derived from different plant species (Reynold, 1996). All the essential oils were active against both gram positive and gram negative bacteria used in this study with MIC regimes in the range of 0.5 to 1.5 mg/ml (w/v).

The antibiotics used such as rifampicin, chloramphenicol, tetracycline, streptozotocin, norfloxacin, amoxycillin, actinomycin D and penicillin produced the strongest

Table 1. Essential oils composition of plant extracts from *Pteronia incana, Artemisia afra, Rosemary officinalis, Lavendula officinalis* and *Tagetes minuta*.

Pecentage composition of essential oils analyses by GC-MS															
Pteronia incana			*Artemisia afra*			*Rosemary officinalis*			*Lavendula officinalis*			*Targetes minuta*			
RT/min	Compound	Peak %	RT/min	Compound	Peak %	RT/min	Compound	Peak %	RT/min	Compound	Peak %	RT/min	Compound	Peak %	
5.29	P-cymene	19.15	5.04	1,8-cineole	2.40	5.80	γ - terpinene	0.24	6.12	Cis-Linalool oxide	0.93	5.14	Delta-4- carene	30.1	
5.51	2(E)(pentenyl)-buten-1,4-iol	0.28	5.78	1,5 heptadien-4-one	0.59	6.31	terpinolene	0.47	7.14	Linalool L	1.52	5.53	dihydrotagetone	14.1	
5.87	Cis-sabinene Hydrate	0.69	7.48	β-Thujone	30.30	6.57	α- terpinolene	0.61	8.22	Camphor	16.28	6.33	-Naginaten	2.30	
6.25	1-undecene	1.63	7.71	Hex-3-en-2-one	4.36	8.45	Camphor	3.85	8.81	Endo-borneol	8.85	7.01	ocimene	5.03	
6.48	Furan	1.85	8.24	Camphor	14.42	8.85	Borneol	3.17	9.53	Verbenone	1.32	7.56	Tagetone	7.30	
7.69	L-camphor	7.54	8.41	L-Borneol	1.76	8.94	Ethanone	0.572	9.77	Bornyl formate	2.16	7.84	Mevalonic lactone	1.80	
8.03	Heptan-3-one	3.88	8.81	Cyclohexene	1.30	9.21	α-terpineol	2.34	10.10	Cyclohexen-1-one	1.72	8.15	Terpinene-4-ol	3.05	
8.37	Terpinene-4-ol	2.29	9.23	Heptane	4.05	9.68	Verbenone	1.89	10.51	Octadiene	0.70	8.43	Camphene	0.87	
8.92	Myrtenol	6.90	9.62	Bornyl formate	1.37	11.20	Bornyl acetate	4.85	12.45	Limonen	1.25	8.81	Octylacetat	1.44	
9.21	Verbenone	1.59	10.04	Carvotanacetone	0.78	11.88	Methylenecyclohexanol	0.31	14.84	Nonan-1-one	0.91	9.42	Phenol	5.70	
9.34	Trans carveol	1.58	10.21	Piperitone	0.61	12.71	Heptenal	0.23	16.31	α-amorphen	1.10	9.85	2-ethylbutan	2.40	
9.91	2-cyclohexen-1-one	1.94	11.33	Phenol	1.16	12.96	α- ylangene	0.73	18.00	Caryophllene oxide	1.34	10.46	Cyclohexen-1-one	4.6	
10.84	L-bornyl acetate	1.35	12.88	Nonane	1.50	13.28	Verbenol	0.32	_	_	_	10.67	4-pyridinol	0.98	
11.73	Junipene	1.54	13.26	Cyclo hexene	0.57	14.26	β-Caryophyllene	3.26	_	_	_	11.10	dihydrocarveol	2.43	
13.75	eugenol	2.77	13.66	3-penten-2-one	0.47	15.20	Cycloundecatriene	4.67	_	_	_	11.63	1-penten-3-ol	0.89	
14.87	α-humulene	1.40	15.59	Diepoxy-p-methane	1.33	15.57	AR-Curcumene	0.50	_	_	_	12.09	2-Cyclohexen-1-one	0.80	
15.53	AR- curcumene	1.78	16.57	Tetrahydroionone	0.43	16.16	β-bisabolene	0.55	_	_	_	12.52	l-lysine	0.89	
16.29	Naphthalene	1.13	17.87	Spathulenol	0.47	16.50	Cis-Calamenene	0.36	_	_	_	13.03	Isomethylionone	0.47	
18.05	Spathulenol	4.41	_	_	_	16.96	α- Calacorene	0.18	_	_	_	13.66	3-heptanone	0.25	
18.26	Farnesene	0.78	_	_	_	17.96	Caryophyllene oxide	1.64	_	_	_	13.42	Hexanoic acid	0.91	
18.60	Zingiberenol	0.38	_	_	_	18.26	α- Pinene	0.23	_	_	_	14.57	Hexadecen-1-ol	1.52	

antibacterial effect compared to netropsin and kanamy-cin. The results of antibacterial test of antibiotics used in this study showed broad spectrum activities at much lower MIC compared to that of the essential oil. *L. monocytogenes, P. flourescens, E. coli* and *K. pnuemoniae* were less susceptible to the treatment of rifampicin at a concentration of 0.1 mg/ml while others were more susceptible between the range of 0.04 and 0.05 mg/ml.

All the bacteria treated with amoxicillin were highly susceptible (0.025-0.07 g/ml), whereas *P. flourescens, E. coli* and A. faecalis were less susceptible. Netropsin was mostly active against *S. Flexneri and P. flourescens and displayed low activity against A. faecalis*. Actinomycin D, on the other hand was very active against all the bacteria tested except *E. coli.* Similarly, kanamycin was highly active against *P. flourescens* (0.01 mg/ml). Whereas, *P. vulgaris, S. Flexneri* and *E. coli* were less susceptible to its treatment. Other antibiotics studied displayed broad spectrum activity toward the selected bacteria. The effect of antibiotics on gram negative bacteria corroborate with the report of Dormans and Deans (2000).

However, being natural products, the oils have been reported to be much safer than the commercial antibiotics. Another advantage of essential oils was their broad spectrum activities against both gram positive and gram negative bacteria (Mangena and Muyima, 1999).

An important characteristic of essential oils and their components is their hydrophobicity, which enables them to partition the lipids of the bacterial cell membrane and mitochondria, disturbing the cell structures and rendering them more permeable (Denyer and Hugo, 1991). The antibacterial activity of essential oils used in this study might be the presence of phenolic components that are most active and appear to act principally as membrane permeabilizers. Although the antimicrobial properties of essential oils and their components

Table 2. Essential oil composition of plant extract from *Eucalyptus cinerea, Citrus limon, Citrus sinensis, Ocimum basilicum,* and *Eriocephatus punchanlatus* as identified by GC- MS *analysis* in the order of retention time of constituents.

Percentage composition of essential oils analyses by GC-MS														
Eucalyptus cinerea			*Citrus limon*			*Citrus sinensis*			*Ocimum basilicum*			*Eriocephatus punchanlatus*		
RT/min	Compound	Peak %	RT/min	compound	Peak %	RT/min	compound	Peak %	RT/min	compound	Peak %	RT/min	compound	Peak %
5.84	1,8-cineole	77.77	5.93	limonene	23.19	5.51	Limonene	70.16	5.15	1,8 cineole	7.35	5.55	Propanoic acid	30.15
7.095	Fenchol	0.51	5.99	limonene	1.83	6.54	L linalool	3.31	6.42	l. linalool	6.04	5.93	Butenoic acid	1.71
7.69	Trans-pinocarveol	1.35	6.04	1-limonene	5.93	6.95	p-menthadien-1-ol	0.60	7.52	1-camphor	1.62	6.08	2-nonadecanol	1.32
8.45	Terpineol-	1.17	6.12	Beta-ocimene	2.53	7.18	Cis-limonene oxide	1.64	9.83	estragole	68.43	6.48	Methylhex-2-yn-4-ol	3.74
9.02	α-terpineol	4.56	6.86	L linalool	2.92	7.52	Camphor	1.93	10.4	benzaldehyde	0.58	7.05	Butanoic acid	7.75
9.66	Menthadien-1-ol	1.68	7.86	citronella	0.61	7.92	L-Borneol	1.05	10.9	Heptan-2-ol	0.50	8.09	Butenoic acid	6.94
9.96	l-carvone	1.08	11.46	citral	11.79	8.49	α- Terpineol	2.10	11.2	benzene	0.24	8.64	cyclohexane	3.10
10.80	Thymol	0.92	11.69	geranial	8.58	8.81	Decanal	2.63	13.4	cyclohexane	0.71	9.15	Acetic acid	4.80
11.61	2acetylcyclopentanone	0.31	13.55	Neryl acetate	7.06	9.15	L. Carveol	0.84	13.7	Methyl eugenol	1.07	9.43	Hexan-2-one	0.83
12.96	α-copaene	0.24	13.92	Geranylacetate	5.05	9.76	L.Carvone	1.80	14.0	propoxybenzaldehyde	0.37	9.76	1-nonene	2.49
13.22	β- bourbonene	0.45	14.64	Trans-caryophyllene	4.55	10.3	geranial	1.74	14.5	α- zingiberene	3.26	10.49	Linayl anthranilate	4.81
14.04	β-Caryophyllene	0.51	14.74	αBergamotene	0.91	10.7	Bornyl acetate	0.99	14.8	Dimethyl phthalate	0.69	10.95	Butenoate	0.92
14.51	Aromadendrene	0.31	15.21	Humulene	1.34	13.6	dodecanal	0.76	15.57	β- farnesene	0.51	11.5	Octenal	1.92
14.87	Dimethyl phthalate	0.62	15.48	Neryl acetate	0.62	14.8	α-humulene	1.00	16.08	azulene	0.26	12.6	Acetoxylinalool	3.25
15.85	bicyclogermacrene	0.49	16.08	bicyclogermac	0.54	15.7	Valencene	0.56	16.31	α-amorphene	0.79	13.1	Copaene	1.15
16.44	Naphthalene	0.36	16.33	β-bisabolene	1.48	20.3	β-sinensal	0.82	16.95	methylbenzene	0.17	13.2	Octadien-1-ol	1.03
17.90	globulol	0.71	17.50	farnesol	0.34	21.5	α- sinensal	0.23	17.67	Methoxy methylbenzene	1.78	13.5	Pentyl ester	1.04
18.05	Viridiflorol	0.40	18.05	Caryophyllene oxide	1.43	21.8	Benzyl benzoate	1.09	17.90	Caryophylene oxide	0.30	13.7	2-butene	1.55
18.94	Delta-selinene	0.90	19.23	11-methylsqualene	0.65	_	_	_	18.58	Naphthalene	0.40	14.06	1-hydroxylinalool	0.96
19.47	β- eudesmol	2.05	19.53	undecanone	0.38	_	_	_	19.20	Naphthalenol	0.86	15.74	Butane	1.85
20.17	Acrylic acid	0.13	_	_	_	_	_	_	20.21	Carboxaldehyde	0.14	16.10	6-tridecanone	1.50
21.90	Benzyl benzoate	0.96	_	_	_	_	_	_	21.84	Benzyl benzoate	0.54	16.61	Methylanisole	1.87
_	_	_	_	_	_	_	_	_	_	_		16.9	Benzenethiol	1.30
_	_	_	_	_	_	_	_	_	_	_		17.99	Caryophyllene oxide	1.76
_	_	_	_	_		_	_	_	_	_		20.10	α- ylangene	0.63

have been reviewed in the past (Mangena and Muyima, 1999), the mechanism of action of these essential oils have not been studied in great detail.

In conclusion, the results of the present study indicate that many essential oils possess antibacterial activity to both gram negative and gram positive bacteria. The present investigation together with previous studies provides support to the effectiveness of antibacterial properties of the essential oils tested especially in the light of the current trend in finding alternative remedies that are effective against increasing numbers of pathogenic bacteria that are resistant to current antibiotics. However, *in vivo* studies and clinical trials would be needed to justify and further evaluate the potential of essential oils as reliable antibacterial agents. Also, more detailed studies of the mechanism of actions of these oils will be of great help in utilizing their full potential in pharmaceutical, cosmetics and aromatherapy industries.

Table 3. Minimum inhibitory concentration (mg/ml) regime of test essential oils against selected bacteria.

Bacteria	PI	AA	RO	LO	TM	EC	CL	CS	OB	EP
Listeria monocytogenes	0.1	1.0	1.5	1.0	1.0	1.0	1.5	1.0	1.0	1.0
Bacillus cereus	1.0	1.0	1.5	1.0	1.5	1.5	0.5	0.5	0.5	0.5
Streptococcus pyogenes	1.0	0.5	1.0	1.0	1.0	1.0	1.0	1.0	0.5	1.0
Bacillus subtilis	1.0	1.0	1.0	1.0	1.5	1.5	1.0	1.0	1.0	1.0
Proteus vulgaris	1.0	1.0	1.0	0.5	1.0	1.5	0.5	1.0	0.5	0.5
Shigella flexneri	1.5	1.5	1.0	1.0	1.5	1.5	1.0	1.0	1.5	1.0
Pseudomonas flourescense	1.5	1.0	1.0	1.0	1.5	1.0	1.0	1.0	1.0	1.0
Esherichia coli	1.0	1.0	1.5	1.0	1.0	1.5	1.0	1.0	1.5	1.0
Klebsiella pnuemoniae	1.0	0.5	1.5	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Alcaligenes feacalis	1.0	1.5	1.5	0.5	1.0	1.5	1.0	1.0	1.5	1.0

PI, Pteronia incana; AA, *Artemisia afra*; RO, *Rosemary officinalis*; LO, *Lavendula officinalis*; TM, *Tagetes minuta*; EC, *Eucalyptus cinerea*; CL, *Citrus limon*; CS – *Citrus sinensis*; OB, *Ocimum basilicum*; EP, *Eriocephalus punchanlatus*.

Table 4. Minimum inhibitory concentration (mg/ml) regime of test antibiotics against selected bacteria.

Organisms	RF	CH	TC	PE	ST	AM	NFX	NE	ACT	KAN
Listeria monocytogenes	0.10	0.08	0.03	0.04	0.04	0.025	0.04	0.10	0.05	0.5
Bacillus cereus	0.04	0.08	0.03	0.04	0.03	0.040	0.04	0.20	0.04	0.5
Streptococcus pyogenes	0.05	0.03	0.04	0.03	0.03	0.040	0.03	0.20	0.10	0.5
Bacillus subtilis	0.05	0.05	0.04	0.03	0.04	0.035	0.04	0.20	0.03	0.4
Proteus vulgaris	0.05	0.04	0.02	0.02	0.04	0.070	0.04	0.10	0.05	1.0
Shigella flexneri	0.04	0.04	0.02	0.03	0.04	0.040	0.03	0.05	0.05	1.0
Pseudomonas flourescense	0.10	0.08	0.04	0.05	0.05	0.512	0.04	0.05	0.05	0.01
Esherichia coli	0.10	0.03	0.03	0.04	0.02	0.512	0.04	0.20	0.10	0.5
Klebsiella pnuemoniae	0.10	0.08	0.04	0.04	0.02	0.040	0.03	0.10	0.05	0.4
Alcaligenes feacalis	0.05	0.04	0.08	0.03	0.03	0.512	0.03	1.00	0.04	1.0

RF, Rifampicin; CH, Chloramphenicol; TC, Tetracycline; PE, Penicillin; ST, Streptozotocin; AM, Amoxicillin NFX, Norfloxacin; NE, Netropsin; ACT, Actinomycin D; KAN, Kanamycin.

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